BIOCHEMICAL OXIDATION OF DAIRY WASTES. II. COMPARATIVE STUDY OF YEASTS

Microbial oxidation of dairy wastes to reduce stream pollution is under investigation in this laboratory. The use of yeast was suggested by earlier studies, which showed that this type of microorganism has a high and rapid assimilative ability. The soluble carbohydrates and proteins in waste water from the manufacture of sweet potato starch and peanut protein (2) have been readily converted to yeast cells, with a decrease in biochemical oxygen demand (B.O.D.) of the effluent after the separation of the yeast for feed purposes. Yeast has been grown on many fruit wastes (9), and recently production of feed yeast from citrus wastes has reduced the pollution (10). Generally, there has been about 80 per cent reduction in the 5-day B.O.D. after the removal of the yeast. These industrial wastes have a much larger proportion of organic matter than the dilute wastes produced in dairies.

Reduction of the pollution of a dairy waste must be accomplished by the removal of the soluble organic constituents. Yeasts convert some of the solubles to relatively stable microbial cell substances that are removable by natural or mechanical sedimentation. Other solubles are partially or completely oxidized as a source of energy for cell production and for maintenance of growth. Ruchhoft *et al.* (6)(7) reported similar observations when glucose was added to activated sludge.

For application to dilute dairy wastes, a yeast must utilize the lactose and proteins and continue its activities at the low concentration of available nutrients. This report presents preliminary studies made with yeasts grown under vigorous aeration in synthetic dairy waste.

A synthetic waste was produced by dissolving 1 g. of dried skim milk in a liter of water. The resulting solution contained 370 p.p.m. of protein and 500 p.p.m. of lactose; it had a 20-day B.O.D. of about 1,050 p.p.m., a value equal to that of the chemical oxygen demand (C.O.D.) (4). This solution approximated fairly well the effluent from many dairy processing operations. The methods used for studying the course of the oxidation have been reported (4).

Aeration Vessels

Sintered-glass gas washing bottles were used as small aeration vessels (4). In each bottle were placed 400 ml. of 0.1 per cent skim milk solution and 100 p.p.m. of silicone antifoam dispersed in ether. The bottles were then sterilized at 15-lb. steam pressure for 15 min. After inoculation, the bottles were placed in a chamber at 30° C Air, filtered through sterile activated

carbon, was bubbled through the solution at the rate of 400 ml. per bottle per minute. Sufficient bottles were prepared to permit the removal of duplicates at 24-hr. intervals.

Analytical Methods

The contents of one of the duplicate bottles were made to the original 400-ml. volume with water, then mixed and centrifuged. Determinations for lactose, protein, and chemical oxygen demand were made on the clear supernatant liquor. The solution in the second bottle was filtered through glass wool. The insoluble material adhering to the side walls and glass wool was dissolved by warm sodium hydroxide and added to the filtered liquor. After neutralization with sulfuric acid and addition of water to obtain the original 400-ml. volume, the total C.O.D. and protein were determined. The C.O.D. of the constituents was calculated from these results (4).

Cultures

Four yeasts were selected for study —*Saccharomyces fragilis* and *Torulopsis cremoris,* both lactose-fermenting yeasts; *Candida lypolytica,* included because of its possible usefulness for this purpose; and *Torulopsis utilis,* which has been used for production of feed yeast from nondairy wastes.

The cultures used for preparation of the inocula for the aeration experiments were maintained on nutrient agar slants containing beef extract, peptone, and 0.1 per cent of dried skim milk. Roux culture bottles containing 150 ml. of 0.1 per cent skim milk were seeded with the yeast and incubated at 30° C for 65 hr. The bottles were thoroughly shaken to assure uniform dispersion of the cells, and 20-ml. quantities were added as inoculum to each aeration vessel containing 400 ml. of sterile synthetic dairy waste.

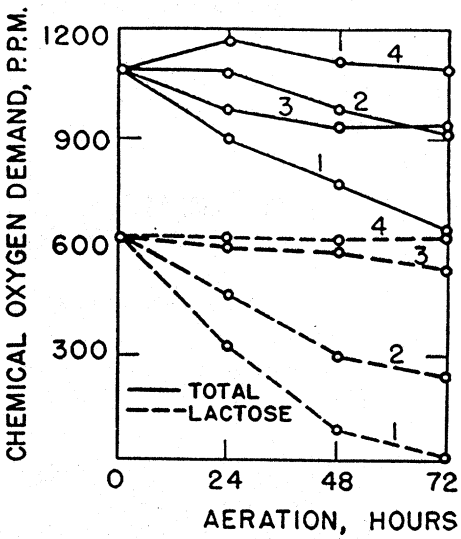

FIGURE 1.—Effects of yeasts on the chemical oxygen demand of dilute milk waste and lactose in the waste. (1) *S. fragilis,* (2) *T. cremoris,* (3) *C. lypolytica, and* (4) *T. utilis*

Selection of an Active Yeast

Figure 1 shows the effects of the four selected yeasts on the C.O.D. of the dilute dairy waste, as well as on the lactose it contained. *S. fragilis* was most active in reducing the C.O.D. of the total waste; the reduction practically paralleling the reduction in C.O.D. of the lactose. For example, the C.O.D. of the total waste was reduced from 1,060 to 630 p.p.m. in 72 hr., whereas the C.O.D. of the lactose was reduced from 640 to 0 p.p.m. In this experiment, there was a conversion of about one-third of the lactose to other substances having an oxygen demand; the remaining two-thirds was completely oxidized, leaving a waste whose total oxygen demand was reduced by 38 per cent. Separation by centrifuge gave a supernatant liquor with a C.O.D. of 200 p.p.m., a reduction of approximately 80 per cent. Of this 200 p.p.m. C.O.D., coagulable protein accounted for 4 p.p.m., nonprotein nitrogenous substances accounted for 112 p.p.m., and other substances for 88 p.p.m. (4).

The results obtained with *T. cremoris* were not so satisfactory, although this organism is an active lactose-fermenting yeast (5). Lactose utilization was not as complete as with *S. fragilis*, and the total C.O.D. was reduced by only 144 p.p.m. Centrifuging gave approximately a 40 per cent reduction in the C.O.D., or half of that obtained by *S. fragilis*, showing that in addition to the sugar, considerable nitrogenous material was still in solution.

The remaining two yeasts had practically no effect on the lactose. Even in the centrifuged liquid, the C.O.D. was only slightly reduced, indicating little cell production or conversion to insoluble material that could be separated.

Figure 2 shows the reduction of C.O.D. in the centrifuged effluent. These results indicate that *S. fragilis* may have possibilities as a means of reducing the pollution of dilute dairy waste.

Supplementing with Ammonium Sulfate

Although casein itself is generally utilized by yeast for growth (8), addition of readily available nitrogen may hasten sugar assimilation. This was indicated by analysis which showed that practically all the protein-nitrogen in the milk waste had been taken up by *S. fragilis* in the first 24-hr. period, although considerable lactose remained. The nitrogen requirement was determined from the average carbon and nitrogen contents of dry yeast (46.8 and 9.75 per cent, respectively) (1)(3) and of the dry skim milk (39.6 and 6.0 per cent). To convert all the carbon in 1 g. of skim milk to yeast cell substance, 82.5 mg. of nitrogen would be required. Of this amount, 60 mg. of nitrogen are present in the milk, thus requiring the addition of 22.5 mg. for total conversion. Aerators were prepared containing synthetic dairy waste to which were added increments of 2.25 mg. of nitrogen as ammonium sulfate. Addition of 4.5 mg., or 11.25 p.p.m., gave the best results. Apparently, all the carbon was not used for cell production. Figure 3 shows that the super-

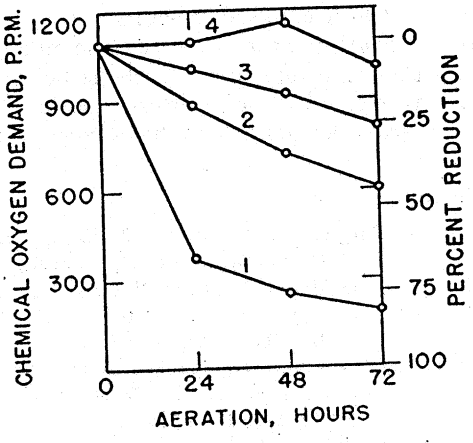

FIGURE 2.—Chemical oxygen demand of the clear effluent of dilute milk wastes after removal of yeasts by centrifuge. (1) *S. fragilis*, (2) *T. cremoris*, (3) *C. lypolytica*, *and* (4) *T. utilis*.

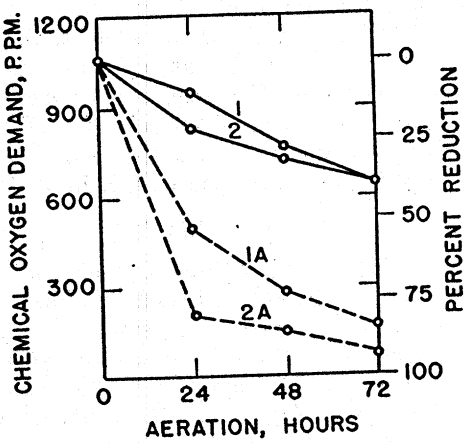

FIGURE 3.—Effect of ammonium sulfate on the chemical oxygen demand of dilute milk waste inoculated with *S. fragilis*.
(1) Ammonium sulfate not added.
(2) 11.25 p.p.m. of nitrogen as ammonium sulfate.
(1A) Supernatant of (1).
(2A) Supernatant of (2).

natant centrifuged solution gave practically the same C.O.D. in 24 hr. when nitrogen was added as in 72 hr. without addition of nitrogen. The lactose had practically disappeared. When nitrogen was added, there was a 93 per cent reduction in the C.O.D. in 72 hr.

Use of Other Salts

Addition of dipotassium phosphate to the waste had no beneficial effect; it seemed to retard the yeast. Sodium nitrate was not utilized by the yeast. Replacement of ammonium hydroxide for the sulfate was not satisfactory, especially in the earlier periods of growth. However, a combination of ammonium hydroxide and sulfate may aid in controlling the acidity, which was pH 4 or lower when sulfate was used.

Rate of Air Flow

In these experiments, a high rate of air flow (equal to one volume per minute or 400 ml. of air per 400 ml. of solution) was arbitarily chosen. The air supplies oxygen to the solution for the maintenance of biological activity; it washes out volatile impurities and waste products, such as carbon dioxide; and it maintains the waste and the yeast in constant agitation, thus favoring increased activity. Experiments made to determine whether a lower rate of air flow would serve these functions showed that similar results were obtained even if the air flow was reduced to one-half or one-fourth this rate.

Discussion

Use of a yeast to reduce the pollution of dilute dairy waste was suggested because of the ability of these organisms to utilize carbohydrates and proteins, the two major soluble constituents of the waste. Under proper conditions of aeration and agitation, the greater part of the soluble lactose and casein were converted to insoluble cell material. When the yeast was removed by centrifuging, the C.O.D. of the effluent was reduced as much as 84 to 93 per cent. In one experiment, after 48 hr. of aeration, the clear effluent had a C.O.D. of 138 p.p.m. and a 5-day B.O.D. of 86 p.p.m.

Only a limited number of yeasts can ferment or utilize lactose. *S. fragilis* has the ability to use lactose and casein at the low concentrations present in dilute dairy wastes. A small amount of ammonium sulfate had an accelerating action. The rate of aeration was reduced to one-fourth volume of air per volume of solution per minute with no apparent change in activity. As only the oxygen dissolved in the waste supports biological activity, apparently at the lower rates of flow there was sufficient oxygen for this purpose as well as for agitation and purging of the liquid.

The feasibility of using *S. fragilis* in a continuous process for the purification of dilute dairy waste is being investigated on a laboratory scale. The recovered yeast may have some value.

References

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